MISCELLANEOUS #350

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street
New York 17, New York

APPLICATION FOR RESEARCH GRANT

Date: April 26, 1962

1. Name of Investigator: PAUL G. MAHLBERG, PH.D.

2. Title: Assistant Professor in Botany

3. Institution & Address: Biological Sciences
University of Pittsburgh
Pittsburgh 13, Pennsylvania

4. Subject: "NICOTINE CONTENT IN ALBINO AND GREEN GENETIC STRAINS OF NICOTIANA TABACUM L. IN TISSUE CULTURE".

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5. Detailed Plan of Procedure: Rate of nicotine production is interpreted to be an inherited trait controlled by specific gene locus under which conditions high nicotine content is dominant over low nicotine content (Valleau, 1949).

In this study highly isogenic strains of known genic composition for the trait of albinism and chlorophyll pigmentation will be analyzed for nicotine content. Comparative analyses will be made upon normal plants of each strain and abnormally proliferated callus derived from these strains. The data will ascertain: 1) if the gene loci under study are associated with nicotine production in the normal plant; and 2) if gene mutations can occur in proliferated callus leading to altered rates of nicotine production in the strains.

Genetic strains of N. tabacum possessing the Mendelian character of albinism in the ratio of: one homozygous green; two heterozygous green; and one homozygous albino have been established in tissue culture. In addition another strain, Cuba White, also has been developed in culture. Albinism in tobacco is controlled by duplicate factors designated Ws_1 located on the G chromosome and Ws_2 located on the T chromosome. The genetic composition of the above isogenic strains relative to pigmentation is: a) albino, $ws_1ws_1ws_2Ws_2$; b) heterozygote, $ws_1ws_1ws_2ws_2$; c) homozygote, $ws_1ws_1Ws_2Ws_2$ and d) Cuba White, $Ws_1Ws_1Ws_2Ws2$. These related strains differ by a single, one pair or two pairs of genes. Plants of these strains will be cultured under sterile conditions to produce normal plants or proliferated callus masses (Venketeswaran and Mahlberg, 1962). The basal culture medium will include (mg/l): NH_4NO_3, 800; $Ca(NO_3)_2.4H_2O$,100; KNO_3,80; KCI,65; KH_2PO_4, 300; $MgSO_4.7H_2O$, 35; $ZnSO_4.7H_2O$,0.1; H_3BO_3,0.1; $MnSO_4.H_2O$,0.01; $CuSO_4.5H_2O$,0.003; $ALCl_3$,0.003; $NiCl_2.6H_2O$,0.003;Kl,0.001; $FeCl_3.6H_2O$,0.1; sucrose, 30,000. The medium is solidified with 7 gm./liter Difco

agar and adjusted to a pH of 5.5. The above medium is supplemented as follows (per liter) for induction of proliferation: 2,4-dichlorophenoxyacetic acid, 1×10^{-6}M (2,4-D), indole-3-acetic acid, 1×10^{-6}M (IAA) and yeast extract, 1 gm. (Y.E.). The cultures will be maintained in a culture room at 25 ± 1°C. with illumination for 12 hours a day with fluorescent lamps. The cultures of normal plants and the proliferated callus tissues derived from the different strains will be analyzed for nicotine content employing the techniques of Dawson (1953).

This investigation can provide data on the relative levels of alkaloid production in the several genetic strains. A primary point of interest in this study will be to determine whether nicotine production will remain constant in the several strains of abnormally proliferated callus tissues over a period of time.

In a potential future extension of this study, it may be possible to analyze chemical factors which will permanently alter the genic composition of callus cells resulting in lowered alkaloid production. It then will be of interest to determine if the plants induced to form from such callus, employing techniques now at our disposal, will retain the depressed level of alkaloid production.

6. Budget Plan:

a.	Salaries	$7,738.00
b.	Expendable Supplies	362.00
c.	Permanent Equipment	---
d.	Overhead (15% of a,b,e)	1,245.00
e.	Other (Travel)	200.00
	TOTAL	$9,545.00

7. Anticipated Duration of Work: September 1, 1962 - August 31, 1963.

8. Facilities and Staff Available: Culture facilities and equipment are available. I shall secure a research assistant to assist in this project.

9. Additional Requirements: ------

10. Additional Information (including relation of work to other projects and other sources of supply):

The present proposal is a result of techniques whereby strains of tobacco can be successfully established in tissue culture. The initial studies have been prepared for publication (Venketeswaran and Mahlberg, 1962).

Recently I have accepted an invitation to join the faculty of the Botany Department at the University of California (Berkeley) for a nine-month period. I plan to carry out this program while at Berkeley as well as at the University of Pittsburgh.

Currently I am receiving support from the National Institutes of Health (Cancer Institute) grant number C-5714, entitled, "Growth rate analysis of suspended plant cell cultures", in which the cells isolated from callus tissues are being evaluated for morphogenetic potentialities; and, an Institutional Grant from the American Cancer Society, entitled, "Cine-phase observations upon growth phenomena of isolated plant cells", in which the response of single cells subjected to diverse cultural conditions are being analyzed cinemicrographically.

Dawson, R. F. Alkaloid biogenesis. IV. The non-availability of nicotinic acid-carboxyl-C^{14} and its ethyl ester for nicotine biosynthesis. Jour. Amer. Chem. Soc. 75: 1953.

Mahlberg, P. G. Isolation of a floating cell strain from submerged cell cultures pf Euphorbia marginata. Exp. Cell Res. 26: 1962.

Mahlberg, P. G. and S. Venketeswaran. Phase cinemicrographic observations on cultured cells.

Signature PAUL G. MAHLBERG
Director of Project

MAX A. LAUFFER
Business Officer of the Institution